ct Information

)ject Name	fas ligand
)ject Code	HG03500
)ject Status	Function
; Code	25750
Clone ID	HTPAN08
Library	Human Pancreas Tumor
Patent Status	In Progress
PTO Serial #	
Created By	Steve Ruben
Date Created	2/2/94
Date Modified	4/14/95

from data
4-28-95

General Comments

Fas is in the TNF superfamily. The Fas antigen (Fas) belongs to the tumor necrosis factor (TNF)/nerve growth factor receptor family, and it mediates apoptosis. Fas ligand is expressed in activated splenocytes and thymocytes, consistent with its involvement in T cell-mediated cytotoxicity and in several nonlymphoid tissues, such as testis.
Mice homozygous for lpr (lymphoproliferation) or gld (generalized lymphoproliferative disease) develop lymphadenopathy and suffer from autoimmune disease. The lpr mice have a mutation in a cell-surface protein, Fas, that mediates apoptosis.

This protein has been difficult to express in both baculovirus and E. coli. New constructs have been made using alternate methionine residues and there is a high expression level with the new construct in E. coli.

Potential Medical Application

Mice homozygous for lpr (lymphoproliferation) or gld (generalized lymphoproliferative disease) develop lymphadenopathy and suffer from autoimmune disease. The lpr mice have a mutation in a cell-surface protein, Fas, that mediates apoptosis. This suggests the important roles of the Fas system in development of T cells as well as cytotoxic T lymphocyte-mediated cytotoxicity.

Patent Information

No patents were found on Fas ligand however it is a member of the TNF superfamily for which there are numerous patents.

Nucleotide BLAST Analysis

Nucleotide Blast of HTPAN08 Full Contig + Screens

Project Information

Project Name	fas ligand
Project Code	HG03500
Project Status	Function
HGS Code	25750
Clone ID	HTPAN08
Library	Human Pancreas Tumor
Patent Status	In Progress
PTO Serial #	
Created By	Steve Ruben
Date Created	2/2/94
Date Modified	4/14/95

from Dept
dated 4-28-95

General Comments

Fas is in the TNF superfamily. The Fas antigen (Fas) belongs to the tumor necrosis factor (TNF)/nerve growth factor receptor family, and it mediates apoptosis. Fas ligand is expressed in activated splenocytes and thymocytes, consistent with its involvement in T cell-mediated cytotoxicity and in several nonlymphoid tissues, such as testis.
Mice homozygous for lpr (lymphoproliferation) or gld (generalized lymphoproliferative disease) develop lymphadenopathy and suffer from autoimmune disease. The lpr mice have a mutation in a cell-surface protein, Fas, that mediates apoptosis.

This protein has been difficult to express in both baculovirus and E. coli. New constructs have been made using alternate methionine residues and there is a high expression level with the new construct in E. coli.

Potential Medical Application

Mice homozygous for lpr (lymphoproliferation) or gld (generalized lymphoproliferative disease) develop lymphadenopathy and suffer from autoimmune disease. The lpr mice have a mutation in a cell-surface protein, Fas, that mediates apoptosis. This suggests the important roles of the Fas system in development of T cells as well as cytotoxic T lymphocyte-mediated cytotoxicity.

Patent Information

No patents were found on Fas ligand however it is a member of the TNF superfamily for which there are numerous patents.

Nucleotide BLAST Analysis

Nucleotide Blast of HTPAN08 Full Contig + Screens

Query= HTPAN08XX HGS #285507
(1863 letters, both strands)

Database: nt
162,249 sequences; 174,644,254 total letters.
Searching.....done

Sequences producing High-scoring Segment Pairs:		High Score	Smallest Poisson Probability P(N)	N
gb|X55448|HSG6PDGEN	Human complete G6PD gene for glucose-...	1029	2.9e-104	2
gb|K03021|HUMTPA	Human tissue plasminogen activator (t...	830	2.0e-100	2
gb|M26434|HUMHPRTB	Human hypoxanthine phosphoribosyltran...	830	4.6e-94	2
gb|T10601|T10601	hbc778 Homo sapiens cDNA clone hbc778...	1140	9.2e-87	1
gb|D00591|HUMRCC1	Human RCC1 gene, complete cds.	577	3.3e-85	3
gb|X69907|HSATPCP1	H.sapiens gene for mitochondrial ATP ...	618	2.3e-84	3
gb|M79078|M79078	EST01226 Homo sapiens cDNA clone HHCP...	1099	1.8e-83	1
gb|X68793|HSAT3	H.sapiens gene for antithrombin III	633	5.2e-83	2
gb|Z15027|HSHLA1467	H.sapiens HLA class III DNA	829	1.2e-82	2
gb|L10641|HUMVITDBP	Human vitamin D-binding protein (GC) ...	721	1.4e-81	2

>gb|X55448|HSG6PDGEN Human complete G6PD gene for glucose-6-phosphate dehydrogenase >gb|Z29527|HSG6PHDH H.sapiens G6PD gene for glucose-6-phosphate dehydrogenase
Length = 52,173

Plus Strand HSPs:

Score = 1029 (284.3 bits), Expect = 4.0e-75, P = 4.0e-75
Identities = 245/294 (83%), Positives = 245/294 (83%), Strand = Plus

```
Query:  1570 TAAAAGATCGCAGTTTGCCTGGTGCAGTGGCTCACACCTGTAATCCCAACATTTTGGGAA 1629
             |||||  |    | ||  ||  || |||||||||||||| |||||||||||  || ||||||
Sbjct:  5032 TAAAATACAAAAATTGGCTGGGCGCAGTGGCTCACATCTGTAATCCCAGCACTTTGGGGG 5091

Query:  1630 CCCAAGGTGGGTAGATCACGAGATCAAGAGATCAAGACCATAGTGACCAACATAGTGAAA 1689
             |||||||||| |||||||  ||  |||||||||| |||||||  ||  |||||||  ||||||
Sbjct:  5092 GCCAAGGTGGGCAGATCACAAGGTCAAGAGATCGAGACCATCCTGGCCAACATGGTGAAA 5151

Query:  1690 CCCCATCTCTACTGAAAGTGCAAAAATTAGCTGGGTGTGTTGGCACATGCCTGTAGTCCC 1749
             |||||||||||||  |||  | ||||||||||||||||  |||  |||   | ||||||||||||
Sbjct:  5152 CCCCATCTCTACTAAAAATACAAAAATTAGCTGGGCGTGGTGGTGCGTGCCTGTAGTCCC 5211

Query:  1750 AGCTACTTGAGAGGCTGAGGCAGGAGAATCGTTTGAACCCGGGAGGCAGAGGTTGCAGTG 1809
             |||||||    |||||||||||| |||||||  |||||  | |||||  ||||||||||||||
Sbjct:  5212 AGCTACTCAGTAGGCTGAGGCAGTAGAATCGCTTGAATCAGGGAGTCAGAGGTTGCAGTG 5271

Query:  1810 TGGTGAGATCATGCCACTACACTCCAGCCTGGCGACAGAGCGAGACTTGGTTTC 1863
```

```
                | |||||||  ||||||  ||||||||||||||||||||||||||||  || ||
Sbjct:  5272 AGCTGAGATCGCGCCACTGCACTCCAGCCTGGCGACAGAGCGAGACTCTGTCTC 5325
```

Score = 133 (36.8 bits), Expect = 1.9, P = 0.85
Identities = 53/86 (61%), Positives = 53/86 (61%), Strand = Plus

```
Query:  1569 CTAAAAGATCGCAGTTTGCCTGGTGCAGTGGCTCACACCTGTAATCCCAACATTTTGGGA 1628
             || ||| |    | || ||| ||  | ||||  | | ||||||||||||| |   ||||||
Sbjct: 34625 CTCAAAAAAAAAAATTAGCCAGGCACGGTGGTGCGCGCCTGTAATCCCAGCTACTTGGGA 34684

Query:  1629 ACCCAAGGTGGGTAGATCACGAGATC 1654
               |  |||  ||   |||  |  || |
Sbjct: 34685 GGCTGAGGCAGGAGAATCGCTTGAAC 34710
```

Score = 126 (34.8 bits), Expect = 0.0019, Poisson P(2) = 0.0019
Identities = 50/81 (61%), Positives = 50/81 (61%), Strand = Plus

```
Query:  1568 ACTAAAAGATCGCAGTTTGCCTGGTGCAGTGGCTCACACCTGTAATCCCAACATTTTGGG 1627
             || |||| |    |  | ||  ||  |   |||  ||  |||||||||||| |  ||||||
Sbjct:    53 ACAAAAAAAAAAAAAAATAGCTGGGAGTGATGGTGCATGCCTGTAATCCCAGCTATTTGGG 112

Query:  1628 AACCCAAGGTGGGTAGATCAC 1648
              || |  |||  ||   ||| |
Sbjct:   113 AAGCTGAGGCAGGAGAATCGC 133
```

Minus Strand HSPs:

Score = 888 (245.4 bits), Expect = 2.3e-63, P = 2.3e-63
Identities = 220/273 (80%), Positives = 220/273 (80%), Strand = Minus

```
Query:  1854 TCTCGCTCTGTCGCCAGGCTGGAGTGTAGTGGCATGATCTCACCACACTGCAACCTCTGC 1795
             | ||||   |  | |  || |||||| |||||| ||||||||   ||||||||||||  |
Sbjct: 21011 TTTCGCCAGGCTGGCGTGCAGGAGTGCAGTGGCGTGATCTCAGTTCACTGCAACCTCCAC 21070

Query:  1794 CTCCCGGGTTCAAACGATTCTCCTGCCTCAGCCTCTCAAGTAGCTGGGACTACAGGCATG 1735
             | || ||| ||||  |||||||||||||||||||| ||||||||||||||||||||||
Sbjct: 21071 CACCTGGGGTCAAGTGATTCTCCTGCCTCAGCCTCCCAAGTAGCTGGGACTACAGGCACC 21130

Query:  1734 TGCCAACACACCCAGCTAATTTTTGCACTTTCAGTAGAGATGGGGTTTCACTATGTTGGT 1675
              |||| ||||| ||||||||||||| | ||| ||||||||||||||||||.||||||||
Sbjct: 21131 CGCCAGCACACACAGCTAATTTTTGTATTTTTAGTAGAGATGGGGTTTCACCATGTTGGT 21190

Query:  1674 CACTATGGTCTTGATCTCTTGATCTCGTGATCTACCCACCTTGGGTTCCCAAAATGTTGG 1615
              ||  |||||||  |||||||| | ||||||| |||| ||| || ||| |||| || |||
Sbjct: 21191 CAGGATGGTCTCTATCTCTTGACCCCGTGATCCACCCGCCTAGGCTTCTCAAAGTGCTGG 21250

Query:  1614 GATTACAGGTGTGAGCCACTGCACCAGGCAAAC 1582
              |||||||||   |||||||  |||||  ||  ||
Sbjct: 21251 GATTACAGGCAAGAGCCACCGCACCCAGCCCAC 21283
```

```
 Score = 878 (242.6 bits), Expect = 1.5e-62, P = 1.5e-62
 Identities = 218/271 (80%), Positives = 218/271 (80%), Strand = Minus

Query:  1856 AGTCTCGCTCTGTCGCCAGGCTGGAGTGTAGTGGCATGATCTCACCACACTGCAACCTCT 1797
              ||||||  ||||||||||||||||||||||  |||||||||  |||||  |  ||||||||||||
Sbjct: 35428 AGTCTCACTCTGTCGCCAGGCTGGAGTGCAGTGGCATGGTCTCAGCTAACTGCAACCTCC 35487

Query:  1796 GCCTCCCGGGTTCAAACGATTCTCCTGCCTCAGCCTCTCAAGTAGCTGGGACTACAGGCA 1737
              |||  |||  |||||||  ||||||||||| ||||||||  ||||||||||| ||||||
Sbjct: 35488 GCCACCCAGGTTCAACTGATTCTCCTGCTTCAGCCTCCTGAGTAGCTGGGATTACAGGTG 35547

Query:  1736 TGTGCCAACACACCCAGCTAATTTTTGCACTTTCAGTAGAGATGGGGTTTCACTATGTTG 1677
               | ||||  ||  |||  ||||||||||| | ||||  ||||||  |||||||||  ||  ||
Sbjct: 35548 CGCGCCACCATGCCCGGCTAATTTTTGTATTTTCTGTAGAGGCAGGGTTTCACCATCTTT 35607

Query:  1676 GTCACTATGGTCTTGATCTCTTGATCTCGTGATCTACCCACCTTGGGTTCCCAAAATGTT 1617
              ||||   ||||||  ||  |  |||  |||  ||||||||||  |||  ||  |||  |||  |  |
Sbjct: 35608 GTCAGGCTGGTCTCGAACACCTGACCTCATGATCTACCCGCCTCGGCCTCCTAAAGTTCT 35667

Query:  1616 GGGATTACAGGTGTGAGCCACTGCACCAGGC 1586
              ||||||||||| ||||||||||||  ||  |||
Sbjct: 35668 GGGATTACAGGCGTGAGCCACTGCGCCCGGC 35698

 Score = 731 (202.0 bits), Expect = 2.9e-104, Poisson P(2) = 2.9e-104
 Identities = 191/247 (77%), Positives = 191/247 (77%), Strand = Minus

Query:  1863 GAAACCAAGTCTCGCTCTGTCGCCAGGCTGGAGTGTAGTGGCATGATCTCACCACACTGC 1804
              ||  ||  |||||  |        |||||||  |||||  |||||||  |||||  |  ||||||
Sbjct: 24275 GAGACAGAGTCTTGTCTGTCACCCAGGCTTGAGTGCAGTGGCACAATCTCGGCTCACTGC 24334

Query:  1803 AACCTCTGCCTCCCGGGTTCAAACGATTCTCCTGCCTCAGCCTCTCAAGTAGCTGGGACT 1744
              |||||  |  |||||  |  ||  ||  ||||||||||  ||||||||||  |  |||  ||||||  |
Sbjct: 24335 AACCTTCGTCTCCCAGATTAAAGCGATTCTCCTCCCTCAGCCTCCCGAGTCACTGGGATT 24394

Query:  1743 ACAGGCATGTGCCAACACACCCAGCTAATTTTTGCACTTTCAGTAGAGATGGGGTTTCAC 1684
              |||||  ||  |||||  |||  ||||||||||||||  |  |||  |||||||  ||||||||||
Sbjct: 24395 ACAGGTATATGCCACCACGCCCAGCTAATTTTTTTATTTTTAGTAGAGGTGGGGTTTCAC 24454

Query:  1683 TATGTTGGTCACTATGGTCTTGATCTCTTGATCTCGTGATCTACCCACCTTGGGTTCCCA 1624
              |||  ||||  ||  |  ||||||  ||  |||  |||  |||||||||  |||||||  ||  |||||
Sbjct: 24455 TATATTGGCCAGTCTGGTCTCGAACTCCTGACCTCGTGATCCGCCCACCTCGGCCTCCCA 24514

Query:  1623 AAATGTT 1617
              ||  ||  |
Sbjct: 24515 AAGTGCT 24521
```

Protein BLAST Analysis

Protein Blast of HTPAN08 Full Contig + Screen

Query= HTPAN08XX HGS#285507
(1863 letters)

Translating both strands of query sequence in all 6 reading frames

Database: nr
113,553 sequences; 31,868,292 total letters.
Searching.....done

Sequences producing High-scoring Segment Pairs:		Reading Frame	High Score	Smallest Poisson Probability P(N)	N
pir|S|A40201	artifact-warning sequence (trans...	+3	241	1.4e-76	3
pir|S|C40201	artifact-warning sequence (trans...	+2	246	7.9e-59	2
pir|S|F40201	artifact-warning sequence (trans...	+3	180	1.1e-20	2
gp|X55777|HSMHCHHS_2	H.sapiens Mahlavu hepatocellular...	+3	190	1.9e-19	1
pir|S|D40201	artifact-warning sequence (trans...	+3	81	2.3e-18	4
gp|L27065|HUMNF2A_1	NF2 gene product [Homo sapiens]	-3	139	7.4e-14	1
gp|L20321|HUMSTK2A_1	protein serine/threonine kinase ...	-1	137	3.0e-12	1
pir|S|E40201	artifact-warning sequence (trans...	+2	98	1.0e-11	3
gp|S58722|S58722_1	X-linked retinopathy protein (3'...	-1	128	1.5e-11	1
pir|S|A46010	X-linked retinopathy protein (C-...	-1	128	1.5e-11	1
gp|M84237|HUMIGTB1A_2	integrin beta-1 subunit [Homo sa...	-3	116	1.9e-10	1
pir|S|A42442	beta 1 integrin subunit, beta 1S...	-3	116	1.9e-10	1
gp|L24521|HUMTRRP_1	transformation-related protein [...	-3	120	1.9e-09	1
gp|K02113|CHKVITB_1	Chicken vitellogenin gene coding...	+3	72	1.0e-07	2
gp|L11672|HUMKRUPZN_1	zinc finger protein [Homo sapiens]	+1	108	1.9e-07	1
gp|X13607|GGVITIIG_1	vitellogenin [Gallus domesticus]...	+3	72	1.1e-06	2
gp|M18060|CHKVITC_1	Chicken vitellogenin gene, compl...	+3	72	1.1e-06	2
gp|U03470|RNU03470_1	ligand for Fas antigen [Rattus n...	+3	84	1.8e-06	2

>gp|U03470|RNU03470_1 ligand for Fas antigen [Rattus norvegicus]
Length = 278

Plus Strand HSPs:

Score = 84 (40.9 bits), Expect = 0.0019, P = 0.0019
Identities = 15/34 (44%), Positives = 23/34 (67%), Frame = +3

Query: 750 GHSFLSNLHLRNGELVIHEKGFYYIYSQIYFRFQ 851
G + +S + + G LVI+E G Y++YS+ YFR Q
Sbjct: 164 GTALISGVKYKKGGLVINEAGLYFVYSKVYFRGQ 197

Score = 66 (32.2 bits), Expect = 1.8e-06, Poisson P(2) = 1.8e-06
Identities = 12/39 (30%), Positives = 22/39 (56%), Frame = +3

Query: 990 YSIYQGGIFELKENDRILVSVINEHLIDMDHEASFFGAF 1106
+S Y G +F L D + V+++ LI+ + +FFG +
Sbjct: 238 HSSYLGAVFNLTVADHLYVNISQLSLINFEESKTFFGLY 276

Full Length Information

Full length sequence of HTPAN08XX HGS# 285507

GGCACGAGCACATTGICTTCTCCAAACTCCAAGAATGAAAAGGCTCTGGGCCGCAAAATAAACTCCTGGGAATCATCAAGGAGTG
GGCATTCATTCCTGAGCAACTTGCACTTGAGGAATGGTGAACTGGTCATCCATGAAAAAGGGTTTTACTACATCTATTCCCAAAC
ATACTTTCGATTTCAGGAGGAAATAAAAGAAAACACAAAGAACGACAAACAAATGGTCCAATATATTTACAAATACACAAGTTAT
CCTGACCCTATATTGTTGATGAAAAGTGCTAGAAATAGTTGTTGGTCTAAAGATGCAGAATATGGACTCTATTCCATCTATCAAG
GGGGAATATTTGAGCTTAAGGAAAATGACAGAATTTGTGGTTTCTGTAACAAATGAGCACTTGATAGACATGGACCATGAAGCCA
GTTTTTTCGGGGCCTTTTTAGTTGGCTAACTGACCTGGAAAGAAAAAGCAATAACCTCAAAGTGACTATTCAGTTTTCAGGATGA
TACACTATGAAGATGTTTCAAAAAATCTGACCAAAACAAACAAACAGAAAACAGAAAACAAAAAACCCTCTATGCAATCTGAGTA
GAGCAGCCACAACCAAAAAATTCTACAACACACACTGTTCTGAAAGTGACTCACTTATCCCAAGAAAATGAAATTGCTGAAAGAT
CTTTCAGGACTCTACCTCATATCAGTTTGCTAGCAGAAATCTAGAAGACTGTCAGCTTCCAAACATTAATGCAATGGTTACATCT
TCTGGCTTTATAATCTACHCCTTGTAAAGACTGTAGAAGAAAACGCAACAATCCATCTCTCAAGTAGTGTATCACAGTAGTAGCC
TCCAGGTTTCCTTAAGGGACAACATCCTTAAGTCAAAAGAGAGAAGAGGCACCACTAAAAGATCGCAGTTTGCCTGGTGCAGTGG
CTCACACCTGTAATCCCAACATTTTGGGAACCCAAGGTGGGTAGATCACGAGATCAAGAGATCAAGACCATAGTGACCAACATAG
TGAAACCCCATCTCTACTGAAAGTGCAAAAATTAGCTGGGTGTGTTGGCACATGCCTGTAGTCCCAGCTACTTGAGAGGCTGAGG
CAGGAGAATCGTTTGAACCCGGGAGGCAGAGGTTGCAGTGTGGTGAGATCATGCCACTACACTCCAGCCTGGCGACAGAGCGAGA
CTTGGTTTCAAAA<AA

HTPAN08xy HGS# 413412
HTPAN08Full lenght edited sequence (with open reading frame)

GGCACGAGCGGCTGCCTGGCTGACTTACAGCAGTCAGACTCTGACAGGTTCATGGCTATGATGGAGGTCCAGGGGGGACCCAGCC
TGGGACAGACCTGCGTGCTGATCGTGATCTTCACAGTGCTCCTGCAGTCTCTCTGTGTGGCTGTAACTTACGTGTACTTTACCAA
CGAGCTGAAGCAGATGCAGGACAAGTACTCCAAAAGTGGCATTGCTTGTTTCTTAAAAGAAGATGACAGTTATTGGGACCCCAAT
GACGAAGAGAGTATGAACAGCCCCTGCTGGCAAGTCAAGTGGCAACTCCGTCAGCTCGTTAGAAAGATGATTTTGAGAACCTCTG

AGGAAACCATTTCTACAGTTCAAGAAAAGCAACAAAATATTTCTCCCCTAGTGAGAGAAAGAGGTCCTCAGAGAGTAGCAGCTCA
CATAACTGGGACCAGAGGAAGAAGCAACACATTGTCTTCTCCAAACTCCAAGAATGAAAAGGCTCTGGGCCGCAAAATAAACTCC
TGGGAATCATCAAGGAGTGGGCATTCATTCCTGAGCAACTTGCACTTGAGGAATGGTGAACTGGTCATCCATGAAAAAGGGTTTT
ACTACATCTATTCCCAAACATACTTTCGATTTCAGGAGGAAATAAAAGAAAACACAAAGAACGACAAACAAATGGTCCAATATAT
TTACAAATACACAAGTTATCCTGACCCTATATTGTTGATGAAAAGTGCTAGAAATAGTTGTTGGTCTAAAGATGCAGAATATGGA
CTCTATTCCATCTATCAAGGGGGAATATTTGAGCTTAAGGAAAATGACAGAATTTTTGTTTCTGTAACAAATGAGCACTTGATAG
ACATGGACCATGAAGCCAGTTTTTTCGGGGCCTTTTTAGTTGGCTAACTGACCTGGAAAGAAAAAGCAATAACCTCAAAGTGACT
ATTCAGTTTTCAGGATGATACACTATGAAGATGTTTCAAAAAATCTGACCAAAACAAACAAACAGAAAACAGAAAACAAAAAAAC
CTCTATGCAATCTGAGTAGAGCAGCCACAACCAAAAAATTCTACAACACACACTGTTCTGAAAGTGACTCACTTATCCCAAGAAA
ATGAAATTGCTGAAAGATCTTTCAGGACTCTACCTCATATCAGTTTGCTAGCAGAAATCTAGAAGACTGTCAGCTTCCAAACATT
AATGCAATGGTTAACATCTTCTGTCTTTATAATCTACTCCTTGTAAAGACTGTAGAAGAAAGCGCAACAATCCATCTCTCAAGTA
GTGTATCACAGTAGTAGCCTCCAGGTTTCCTTAAGGGACAACATCCTTAAGTCAAAAGAGAGAAGAGGCACCACTAAAAGATCGC
AGTTTGCCTGGTGCAGTGGCTCACACCTGTAATCCCAACATTTGGGAACCCAAGGTGGGTAGATCACGAGATCAAGAGATCAAG
ACCATAGTGACCAACATAGTGAAACCCCATCTCTACTGAAAGTGCAAAAATTAGCTGGGTGTGTTGGCACATGCCTGTAGTCCCA
GCTACTTGAGAGGCTGAGGCAGGAGAATCGTTTGAACCCGGGAGGCAGAGGTTGCAGTGTGGTGAGATCATGCCACTACACTCCA
GCCTGGCGACAGAGCGAGACTTGGTTTC

Amino Acid Translation of the HTPAN08 Full length Clone S04:

GTSGCLADLQQSDSDRFMAMMEVQGGPSLGQTCVLIVIFTVLLQSLCVAVTYVYFTNELKQMQDKYSKSGIACFLKEDDSYWDPN
DEESMNSPCWQVKWQLRQLVRKMILRTSEETISTVQEKQQNISPLVRERGPQRVAAHITGTRGRSNTLSSPNSKNEKALGRKINS
WESSRSGHSFLSNLHLRNGELVIHEKGFYYTYSQTYFRFQEEIKENTKNDKQMVQYIYKYTSYPDPILLMKSARNSCWSKDAEYG
LYSIYQGGIFELKENDRIFVSVTNEHLIDMDHEASFFGAFLVG.LTWKEKAITSK.LFSFQDDTL.RCFKKSDQNKQTENRKQKN
LYAI.VEQPQPKNSTTHTVLKVTHLSQENEIAERSFRTLPHISLLAEI.KTVSFQTLMQWLTSSVFTIYSL.RL.KKAQQSISQV
VYHSSSLQVSLRDNILKSKERRGTTKRSQFAWCSGSHL.SQHFGNPRWVDHEIKRSRP..PT..NPISTESAKISWVCWHMPVVP
AT.EAEAGESFEPGRQRLQCGEIMPLHSSLATERDLV

The Amino Acid Translation of the Fas ligand using the first Methionine (HTPAN08S04 51bp ATG):

MAMMEVQGGPSLGQTCVLIVIFTVLLQSLCVAVTYVYFTNELKQMQDKYSKSGIACFLKEDDSYWDPNDEESMNSPCWQVKWQLR
QLVRKMILRTSEETISTVQEKQQNISPLVRERGPQRVAAHITGTRGRSNTLSSPNSKNEKALGRKINSWESSRSGHSFLSNLHLR
NGELVIHEKGFYYTYSQTYFRFQEEIKENTKNDKQMVQYIYKYTSYPDPILLMKSARNSCWSKDAEYGLYSIYQGGIFELKENDR
IFVSVTNEHLIDMDHEASFFGAFLVG.

The Amio Acid Translation of the Fas ligand using a Methionine more 3' (HTPAN08so4 185bp ATG):

MQDKYSKSGIACFLKEDDSYWDPNDEESMNSPCWQVKWQLRQLVRKMILRTSEETISTVQEKQQNISPLVRERGPQRVAAHITGT
RGRSNTLSSPNSKNEKALGRKINSWESSRSGHSFLSNLHLRNGELVIHEKGFYYTYSQTYFRFQEEIKENTKNDKQMVQYIYKYT

SYPDPILLMKSARNSCWSKDAEYGLYSIYQGGIFELKENDRIFVSVTNEHLIDMDHEASFFGAFLVG.

Tissue Distribution

August 31, 1994

The HTPAN08 Eco RI/ XhoI digested fragment was given to Guo-Liang Yu to use as a probe for a northern of tumor and normal tissues.

Protein Expression

FOR THE AMINO ACID TRANSLATION OF THE 2 DIFFERENT FAS LIGAND CONSTRUCTS (51BP ATG AND 185BP ATG) SEE FULL LENGTH INFORMATION>

August 25, 1994

Transcription and Translation was performed using the Promega TNT kit using T3 Polymerase, Rabbit Reticulocyte Lysate, and 35-S methionine. The protein was expressed at about 30kdaltons.

August 30, 1994

Primers were made to express the protein in the baculovirus system using a 5'Bam HI primer and an 3' Asp718 primer.

5' Bam HI: GCG CGG ATC CAC CAT GGC TAT GAT GAT GGA GGT C
3' Asp 718: GCG CGG TAC CAG TTA GCC AAC TAA AAA GGC CCC G

09/02/94

HTPAN08S04 5'Bam HI/3' Asp 718 fragment was PCR'd

95C 5 min
30X of
95 C 20 sec
55 C 20 Sec
72 C 1 Min
72 C 7.5 Min.

9/06/94

The fragment was then digested with Bam HI and Asp 718 separately for 4 hours and digested for an additional 4 hours with the complementary enzyme. The fragment was isolated on a 0.8% Low Melting Point Gel and cleaned-up using GeneClean (from Bio 101).

Ligations were set-up using the pA2 Bacculovirus vector that had been digested with Bam HI and Asp 718 in a 20ul reaction. The ligation was incubated at 16C overnight. Controls of Vector only, fragment only and ligation reaction mix only were also done.

09/07/94

10 ul of the ligation reaction was used to transform chemichally competent DH5-alpha cells that were made here at HGS. 5ng of pA2 Plasmid DNA was used as a positive contol for the transformation.

10 ul of ligation reaction into 100 ul of thawed cells
Incubate on ice 1 hour
Heat to 42 C for 45 seconds
Place on ice
Add 400 ul of LB
Incubate at 37C for 1 hour.
Plate onto LB+ 100ug/ml Ampicillin plates
Incubate plates at 37C overnight.

09/08/94

Inoculate 200ul of LB+ Amp in a Sterile 96 Well Corning dish with individual colonies from plates. Incubate the plate at 37 C with vigorous aeration for 4 hours. PCR to determine if inserts are present. Picked 20 colonnies and of that 17 had the correct size insert. Inoculated 5 ml of TB+Amp with cultures 1-17 for boiling mini preps. Incubate at 37C with aeration.

09/09/94

Do boiling minipreps using STET with RNase and lysozyme. Submitt 1-5 for sequencing using internal primers to confirm the sequence and to make sure that the cloning site remained intact.

09/12/94

The clones all look good. The sequences seem to match well. Inoculate 200 ml of TB+Amp for Qiagen Maxi Prep. Incubate at 37C overnight.

09/13/94

Qiagen Maxi Prep. Recovered @500ug of DNA. Ready to do Transfections.
DNA was given to Jian Ni in Protein expression Department for transfections into stable cell lines.

Primers were made to express the protein in the pD10 expression system- 5' hexa a His tag- cloned into 5'Bam HI and 3' Xba sites.

HTPAN08 5'Bam/ 3' Xba into pD10
New primers were made after a mistake was found in the 5' Bam primer. New primer was also made for the 3' xba site that included an additional stop codon. (11/14/94) After transformation into M15 rep4 cells, 31 positive clones for HTPAN08 were found by PCR screening.

11/16/94 Small scale plasmid prep was done on 10 of the clones and when digested with Bam and Xba, @800bp fragment was cut out. Theses clones were also sequenced with the PD10 5' primer (FASPD01-10 RP01)

11/18/94 A mistake was found in the 5' Bam site for the PA2 expression system. New primers were made and the fragment rePCR'd. A region of high Hydrophobicity was found at the 5'end that might inhibit expression, so 2 primers were made. One at the first Met (@51bp) and one at the first Met after the hydrophobic region (@185bp).

11/21/94 Sequence looked good, the Bam site was conserved as well as the ATG site. small scale inductions will be tried to see if these clones can be induced. PA2 inserts were PCR'd using the new 5'Bam and the old 3' Asp primers.

11/28/94 The inserts are being digested with Bam and Asp.

11/30/94 After doing small scale inductions and running them on a 10% PAGE gel, no visible induction could be seen at @30 kD so a small scale purification over a Nickle sulfate column is being tried. The fragments for the PA2 constructs that were digested with Bam and Asp were isolated on a 0.8% LMP gel and gene cleaned. Ligations were set-up to incubate overnight at 16C.

01/19/95

Small scale inductions of the HTPAN08 185bp + PQE60 constructs showed one clone that induced 4-1A.

01/24/95
A 300ml culture of HTPAN08S04 185bp +PQE60 (4-1A) was induced and purified over a nickle sulfate column (Qiagen) and all fractions were run on a 15% Acrylimide stacking gel and showed a large induced band at about 28 KiloDaltons. The protein is in 5ml of 6Molar Guanidine Hydrochloride.

01/27/95

Ligations were set-p for the HTPAN08 consructs in both the PA2 vector and the PD10 vector using insert PCR'd from the Primers 9111,9112,9113,9114 and 3146. The ligations incubated over the weekend at 16 C.

01/30/95
The ligations were transformed into M15 Rep 4 cells for the PD10 constructs and into DH5-alpha cells for the PA2 constructs. The cells were then plated onto LB+Ampicillin plates and incubated at 37 C overnight.

01/31/95
The transformations all worked well with little or no colonies on the vector alone and fragment alone plates. Clones were picked into LB+amp for PA2 constructs and into LB+amp/Kan for the PD10 constructs. The clones were then incubated at 37 C for 4 hours and PCR'd to check for inserts using the primers 9111,9112,9113,9114 and 3146.

02/01/95
The PCR products were run on a 1% Agarose gel and positive clones were seen. 5 mls of TB +amp were inoculated with the positive clones for the PA2 constructs for mini prep DNA. THe PD10 constructs were inoculated into LB+amp/Kan for mini inductions.

To the 3 mls of protein in 6M GnHCl pH5, 25mls of 6M GnHCL pH8.0 was added and reapplied over a nickle sulfate charged column. The Column was then sent to the protein expression group to have renatured over the column. 2 mls of protein was dialyses in decreasing concentrations of GnHCl to a final concentration of 0 Molar GnHCl in 10% Glycerol, it began with 3 M GnHCl and the concentration decreased over the course of several days

02/03/95
The renatured protein on the column was eluted in 2-2.5ml fractions in Imidizole elution buffer and 50 ul run on a protien gel. Protein looked good and was stored at 4C. Running small scale inductions on the 185bp ATG constructs in PD10 showed

several clones that showed good inductions. Large scales will be done.

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☑ FADED TEXT OR DRAWING
- ☑ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☑ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☑ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.